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1920

A COURSE
OF
PRACTICAL PHYSIOLOGY
FOR
AGRICULTURAL STUDENTS

BY

J. HAMMOND, M.A.

AND

E. T. HALNAN, M.A.

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SCHOOL OF AGRICULTURE,
CAMBRIDGE UNIVERSITY

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M. M. W.

PREFACE

This Course of Practical Work is written primarily for the second-year students taking the Course in Agriculture at the University of Cambridge, but it is hoped that it may also serve as a basis for courses in other Agricultural and Veterinary Colleges.

The chemical side has been reduced to a minimum as this is already provided for to a large extent in *A Course of Practical Chemistry for Agricultural Students*, Vol. II (Newman and Neville).

The general aim throughout the course has been to allow the student to prepare sections for himself of the most important tissues so that he may possess a representative series of slides for subsequent private study. In addition a number of demonstration slides and specimens are exhibited so that should he fail in the preparation of any specimen he may be able to see a typical section. These demonstration slides and specimens also illustrate tissues which the student may have difficulty in preparing for himself or which are considered not to be of sufficient importance to be included in the slides he prepares for himself.

It is suggested that the student should examine the slides under the microscope and should sketch them, preferably in a note book alongside his lecture notes on the subject concerned or in the blank pages provided in this book. In this way the structure may be used as a basis for the study of the physiology of the organ.

The authors wish to express their indebtedness to the following books which are commended to the student as books of reference: *Practical Histology*, J. N. Langley; *Essentials of Histology*, E. S. Schafer; *Exercises in Practical Physiological Chemistry*, S. W. Cole; and *Physiology of Farm Animals*, F. H. A. Marshall.

To Mr F W. Foreman our thanks are due for his helpful criticism of the Chemical exercises, and lastly, we are under great obligations to Dr F. H. A. Marshall for his suggestions and criticisms throughout.

J. H.
E. T .H.

October, 1920.

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INTRODUCTION

Cells. The typical animal cell with its nucleus surrounded by protoplasm forms the basis of the tissues of the body. These cells exhibit a considerable variation in structure due to specialization for particular functions; however as a rule animal cells have no distinct cell wall such as exists in plants. Owing to the fact that animal tissues are usually soft and without cell wall structure special preparations have to be made in order to obtain sections for the study of their minute structure.

Preparation of Sections. The process for the preparation of a section is as follows: small pieces of tissue from a freshly-killed animal are placed in a *Fixing Fluid* which coagulates and hardens the proteins of the tissue.

The most common fixing agent and probably the best for general purposes is a solution of 10 *per cent.* formalin. *Other reagents are frequently used for fixing tissues as some tissues are preserved better by some reagents than by others:* Alcohol (95 *per cent.*) *is frequently used;* Fleming's solution (1 *per cent. chromic acid,* 15 *c.c.*; 2 *per cent. osmic acid,* 4 *c.c.; glacial acetic acid,* ·5 *to* 1 *c.c.*) *is useful for preserving gland tissues.*

Müller's fluid (*potassium bichromate,* 25 *grs.; sodium sulphate* 10 *grs.; water,* 1 *litre*) *is also another useful fixing agent.*

After the tissue has remained in the fixing fluid for several days (the time will depend on the fluid used) it is washed to remove the fixing agent and is then ready for cutting into sections.

Two methods are in general use for *embedding* prior to cutting these sections: (1) the Freezing Method and (2) the Paraffin Method. The object of embedding is to enclose the tissue in a solid mass so that it will not tear or break when the section is being cut. In this solid condition it is also possible to cut sections of definite thickness.

(1) The Freezing Method. *A small piece of the tissue is washed in water to free from the fixing agent, placed for a day or more in a solution of gum arabic and then frozen on the stage of the microtome by the evaporation of ether.*

(2) The Paraffin Method. *After washing in water the tissue is passed through a series of alcohols to remove the water. It is then placed in xylol or some other clearing agent which mixes with both alcohol and paraffin wax. When clear, the tissue is placed in a bath of molten paraffin wax, kept at a temperature of* 45° *to* 60° *C. and after a short time is*

removed and allowed to solidify in the wax. The block of wax is then placed on a microtome and sections are cut.

Use of Stains. Stains are used because their affinity for certain substances enables the various tissues to be differentiated. The following are some of the stains most frequently used:

Haematoxylin (Delafield's) (4 *c.c. saturated solution of haematoxylin in absolute alcohol;* 150 *c.c. of ammonium alum saturated in the cold, leave a week to filter and add* 25 *c.c. methyl alcohol and* 25 *c.c. glycerine*). *Stains nuclei in all tissues a bluish purple colour.*

Eosin (*solution in* 75 *per cent. alcohol*). *Stains muscle, elastic tissue and horny layers of skin a pale pink colour and red blood corpuscles a bright red colour.*

Van Gieson's stain (95 *per cent. alcohol,* 200 *c.c.; picric acid,* 2 *grs.; acid magenta,* 0·2 *gr.*). *Stains white connective tissue a bright magenta and muscle, red blood corpuscles, elastic and horny tissue a pale yellow colour.*

Examination of Slides. Tubular organs such as the intestine or trachea can be cut in section in several ways and the appearance of the section will largely depend on the direction in which the section has been cut.

Before examining any slide under the microscope it should first be observed by the naked eye in order to determine if possible which way the section has been cut and to obtain a general idea of the proportions and extent of the section.

A complete *transverse section* of the intestine would show the section in the form of a tube cut across, while a *longitudinal section* of its wall would be straight and not circular, and would show on the inside the glandular layer and on the outside the muscular coat. Sections are also sometimes cut *parallel to the surface* of the organ. If the tube of the small intestine were slit open and laid out flat sections could be cut parallel to the glandular surface and the first sections so cut would comprise all the glandular tissue while the latter sections would contain the muscular portion of the intestine.

Structures such as tubular glands, which are frequently coiled, may be seen to be cut through several times in one section. In one place the gland may appear to be a straight tube and then a sudden coil causes it to disappear from the section and yet another twist will bring it into the plane of the section again; this time, unless the gland runs exactly in the same plane as the section, it will appear as a circular tube cut across.

I

THE TISSUES

Examine, under both low and high powers of the microscope, and sketch in your note-books the following *tissues* of which demonstration slides have been prepared.

EPITHELIAL TISSUES. The epithelial cells are found covering free surfaces, both internal and external.

1. *Pavement epithelium.* Consists of a layer of flattened cells arranged in a mosaic, hardly visible in cross-section. This epithelium is found lining the interior of blood-vessels, the lungs, lymphatics and the peritoneum.

2. *Ciliated epithelium.* Appears as a single row of cells provided at the free surface with a fringe of hair-like cilia. It occurs chiefly in the Trachea and Fallopian Tubes.

3. *Glandular epithelium.* Glandular epithelium lines the alveoli of secreting glands, and its appearance varies with the amount of secretory substance found in the cell; this substance sometimes quite obscures the nuclei. The cells usually appear cubical or columnar, and are set round the tubular cavity of the gland into which the secretion is poured.

4. *Stratified epithelium.* Note that the cells are arranged in a number of layers. The cells of the deeper layer are columnar and as the surface is approached they become polygonal, while the outermost ones consist of flattened scales. It is from epithelium of this sort that horn and hoof are formed.

CONNECTIVE TISSUES.

5. *Areolar connective tissue.* Note that it consists mainly of bundles of *white fibres* having a wavy course with a few elastic fibres forming an open network and a number of *Lamellar cells* with nuclei. This tissue enters into the composition of most organs of the body forming a framework which supports and connects other tissues.

6. *Elastic fibres.* Besides occurring in the connective tissue these fibres occur largely in the arteries and veins. Note that they run singly and not in bundles, and that they have a curved appearance owing to their recoil due to the contraction of the artery. Normally when under tension they are straight. These fibres stain intensely with certain dyes (orcein).

7. *Lymphoid tissue.* This consists of a network of connective tissue in the meshes of which lymph-corpuscles are held. Note that the lymph-corpuscles are small round cells consisting largely of nucleus.

8. *Adipose tissue.* Note that it consists of fat cells, each of which has a delicate covering with a nucleus pressed to one side. They are of varying size depending on the amount of fat (stained red with Sudan III) deposited in them. These cells occur in the connective tissue in various parts of the body, forming definite layers in certain situations. They stain black when treated with osmic acid.

9. *Cartilage.* This consists of fairly clear cells, often arranged in groups of two or four, disseminated in a solid ground substance or matrix, which is stained slightly with haematoxylin. Cartilage which is commonly called "gristle" is the forerunner of bone in the skeleton and occurs chiefly in connection with it.

Bone, see slide 42, page 79.

MUSCULAR TISSUES.

10. *Unstriated muscle.* Note that the muscle fibres, which often run in bundles, are long cells tapering at the end and have elongated or rod-shaped nuclei. These cells occur in the blood-vessels, intestinal tract, bladder and other parts of the body which have involuntary movement.

11. *Striated muscle.* The long muscular fibres are seen grouped in bundles, some of which run longitudinally, while others are cut transversely. In those which run longitudinally observe the transverse striations of dim and bright bands. Fibres of striated muscles collected in groups form what is commonly known as the flesh of animals and are used in connection with the voluntary movements.

Cardiac muscle, see slide 29, page 55.

NERVOUS TISSUES, see page 75.

II

STAINING AND MOUNTING

Stain and mount sections of Tongue.

The section given has been obtained by the following method: After fixation (in 10 per cent. formalin) the tissue was embedded in paraffin wax and cut into thin sections by means of a microtome. Each section was then placed on a drop of water on a coverslip (previously smeared with egg-white) and gently warmed to flatten the section out The water was then drained off and the section gently blotted and allowed to dry.

Method.

Arrange watch glasses in series containing the following liquids: (1) xylol, (2) absolute alcohol (covered with a watch glass to prevent evaporation and absorption of water from the air), (3) 95 per cent. alcohol, (4) 75 per cent. alcohol, (5) 50 per cent. alcohol, (6) 30 per cent. alcohol, (7) haematoxylin, (8) Van Gieson's fluid (acid magenta and picric acid), (9) water.

Treat the section as follows: place in xylol to dissolve out the paraffin wax. At the stage where this is complete the section will appear quite transparent. By means of needles or forceps transfer the section to 95 per cent. alcohol. *Great care should be taken to drain off all superfluous liquid before transferring the section from one watch glass to another.* Pass down through the series of alcohols and transfer to the haematoxylin stain (stains nuclei) for about 10–15 minutes. Wash in water and place in Van Gieson's stain for 2–3 minutes (stains muscle, horny layers (yellow) and connective tissue (red)). Again wash in water and then dehydrate by passing up through the series of alcohols, allowing 3–5 minutes in each.

Remove the section from absolute alcohol, blot gently and place in xylol until the section becomes clear, showing no opaque spots.

Place the section face upwards on blotting paper and tilt to run off superfluous xylol. Let a small drop of Canada balsam fall on the section, lower a microscope slide on it and press gently on the section until it adheres to the slide. Reverse the slide and examine under the microscope. Identify the various tissues present in the section.

III

THE SALIVARY GLANDS

(A) *Stain and mount sections of the Salivary Gland.*

The method of staining and mounting for this tissue is the same as that adopted for the tongue with this difference, that eosin is used as a stain for the basal tissue instead of Van Gieson's fluid.

After staining with haematoxylin and passing through 30 per cent. and 50 per cent. alcohols, the section should be placed in eosin for about one or two minutes. Transfer it to 75 per cent. alcohol, dehydrate and mount in the same way as was used previously for the tongue. In dehydrating, care should be taken that all the eosin stain is not washed out. The appearance of the section should neither be distinctly red nor distinctly blue; if it is red, leave in the 95 per cent. alcohol until the red colour becomes fainter; if it is blue, stain again in eosin and pass more quickly through the alcohols.

Examine the slide under both low and high powers of the microscope.

(B) *Examine and sketch demonstration slides of:*

12. *Mixed salivary gland.* Note the tubular *alveoli* which when cut across appear round and consist of one layer of polyhedral cells. These cells are of two types:

(1) The *mucous cells,* which are transparent and contain the precursor of mucin. The mucin when secreted in the saliva enables the food to be swallowed readily.

(2) The *serous cells,* which occur at the ends of the alveoli, are filled with small granules which are probably the precursor of the ferment ptyalin of the saliva.

The cells of the gland are often so packed with granules that the *lumen* (or central cavity) of the alveolus is obscured. The *ducts* of the gland through which the secretion passes to the exterior, are seen as a single layer of cells, the inner border of which forms a distinct ring bounding the central lumen.

13. *Resting salivary gland.* Observe that the cells of the gland are large and the nucleus is obscured by the accumulation of the granules which are ready for secretion.

14. *Active salivary gland after prolonged secretion.* Compare with the previous section and observe that the cells are smaller, the nuclei are easy to distinguish and only a few granules are present. These granules are situated round the lumen of the gland.

III.

THE SALIVARY GLANDS

(8) *Make and mount sections of the Salivary Gland*

The method of staining and mounting for this tissue is the same as that adopted for the tongue with this difference, that eosin is used as a stain for the basal layer instead of Van Gieson's fluid.

After staining with haematoxylin and passing through 30 per cent. and 50 per cent. alcohols, the section should be placed in eosin for about one to two minutes. Transfer to 70 per cent. alcohol, dehydrate and mount in the same way as was used previously for the tongue. In dehydrating, care should be taken that all the eosin stain is not washed out. The appearance of the section should neither be distinctly red nor distinctly blue; if it is red, leave in the 95 per cent. alcohol until the red colour becomes fainter; if it is blue, stain again in eosin and pass more quickly through the alcohols.

Examine the slide under both low and high powers of the microscope.

(9) *Examine the stained demonstration slides of:*

11. *Mixed salivary gland.* Note the tubular alveoli which when cut across appear round and consist of one layer of polyhedral cells. These cells are of two types:

(1) The mucous cells, which are transparent and contain the secretion of mucin. The mucin when secreted in the saliva enables the food to be swallowed easily.

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13. *Active salivary gland after prolonged secretion.* Compare with the previous section and observe that the cells are smaller, the nuclei are easy to distinguish and only a few granules are present. These granules are situated round the lumen of the gland.

IV

CHEMISTRY OF CARBOHYDRATES

(A) Carry out the following tests with the solutions of *glucose*, *cane sugar*, and *maltose* provided, recording your results in a table.

(1) Boil with a little sodium hydrate solution (Moore's test).

(2) Treat two or three c.c. of 5 per cent. caustic soda with four or five drops of 1 per cent. copper sulphate solution. Add equal bulk of sugar solution and boil (Trommer's test).

(3) Boil a little Fehling's solution in a test-tube. Add about equal volume of sugar solution and boil again (Fehling's test).

(4) Boil some freshly prepared Barfoed's reagent, and add to it sugar solution drop by drop, boiling the whole time (Barfoed's test).

(5) Examine under a microscope and sketch the crystals of osazone prepared by the demonstrator.

NOTE. (*a*) *Maltose* gives a yellow colour with test (1), and a red-yellow precipitate in tests (2) and (3), but no precipitate in (4) as it has not sufficient reducing power to split up Barfoed's reagent.

(*b*) *Glucose* (*dextrose*) gives the same reactions as maltose, but in addition in test (4) reduces Barfoed's reagent to a slight yellow-red precipitate which may be seen at the bottom of the test-tube.

(*c*) *Cane sugar* gives a negative result with all these tests. If, however, it is first boiled with dilute acids it will break down into dextrose and laevulose, and will then give a positive reaction after neutralisation.

(*d*) Crystals of *glucosazone* have the appearance of fine yellow needles in fan-shaped aggregates, sheaves or crosses.

Lactosazone usually separates as ovoid or spherical clusters of fine needles. Crystals of *maltosazone* are similar to those of glucosazone, but much broader.

(B) Carry out the following tests with the *starch paste* obtained by boiling starch with water, and also with *glycogen* (or animal starch).

(1) Add iodine drop by drop to the starch solution, boil and cool. Record results.

(2) To some starch paste in a test-tube add equal bulk of saturated ammonium sulphate solution. Allow to stand 5 minutes, filter, and test filtrate with iodine. Repeat experiment with glycogen.

(3) Boil 10 c.c. of the starch paste in a small beaker after adding 5 drops of concentrated sulphuric acid, and boil for 7 minutes. Add a drop of litmus solution and neutralise with NaHO, keeping the reaction on the acid side. Divide into two portions. Cool one portion under the tap, and add a drop of iodine solution. Compare the colour obtained with that given by a dextrin solution to which iodine is added. To the other portion add 3 c.c. Fehling's solution and boil.

NOTE. (*a*) In test (1) starch gives a blue colour and glycogen a red one. (2) Starch and glycogen are precipitated by half-saturation with ammonium sulphate and no reaction should be given by the filtrate. (3) Starch by the action of acid becomes broken down into dextrin, which gives a red-brown colour with iodine. On further treatment with acid the dextrin is broken down into glucose which reduces Fehling's solution.

(*b*) Glycogen is obtained in the following manner: A rabbit is well fed with carrots for a few days so that its liver may contain a large store of glycogen derived from the sugar of the food. It is killed and cut open quickly and the liver is plunged into boiling water, minced and ground with sand so that the enzymes converting the glycogen to sugar are destroyed.

V

ENZYMES AND THEIR ACTION ON FOODSTUFFS

(Effect of ptyalin on starch)

(1) Collect about 5 c.c. of your own saliva in a beaker. Test its reaction with litmus paper: it is *alkaline.*

(2) Transfer the saliva to a test-tube, and add strong acetic acid. A stringy precipitate of mucin is formed, which may be removed by stirring with a glass rod, the mucin forming a clump on the end of the glass rod.

(3) Obtain some diluted saliva as follows: warm some distilled water to about 40° C. and with a little thoroughly rinse out the mouth. Now take about 20 c.c. of the warm water into the mouth, and move it about for at least a minute. Collect this in a clean beaker and repeat the process twice more. Thoroughly mix the diluted saliva thus obtained, shake vigorously and filter.

(4) Label two test-tubes *A* and *B*. To *A* add 5 c.c. 1 per cent. starch solution and 5 c.c. diluted saliva; to *B* add 5 c.c. starch solution and 5 c.c. of diluted saliva which you have previously boiled. Place both test-tubes in a water bath at 40° C. Place a series of drops of iodine solution on a white porcelain plate or piece of paper. From time to time transfer by means of a glass rod a drop of the digesting mixture to a drop of iodine. In the case of *A* the blue colour produced at first will later become blue-violet, red-brown and light yellow. When a drop of the mixture gives no further colour with iodine, boil a few c.c. with Fehling's solution. A well-marked reduction is obtained, showing the presence of reducing sugar. Compare with results obtained in the case of test-tube *B*.

NOTE. *The above experiments demonstrate that saliva is an alkaline fluid containing a protein body called mucin, and a starch splitting ferment which is called ptyalin. Starch by its action is converted into the sugar maltose. It also shows that ptyalin belongs to a class of ferments called enzymes which are destroyed by heat.*

Query: How would you ascertain the temperature of destruction of the ferment ptyalin?

VI

THE SIMPLE STOMACH

(A) *Stain and mount sections of* (1) *the Fundus region of the Stomach and* (2) *the Pyloric region of the Stomach.*

Stain with haematoxylin and eosin and examine the slides under the microscope.

(B) *Examine and sketch demonstration slides of*

15. *Oesophagus.* Observe that the section consists of three coats:

(*a*) The outer or *muscular coat* is composed of two layers of muscle fibres, the inner circular and the outer longitudinal. These muscle fibres consist of both the voluntary and involuntary type, and in ruminants the former predominate. The contractions of this coat drive the food through the oesophagus.

(*b*) The connective tissue or *submucous coat* connecting the mucous and muscular coats, contains a few mucous glands, the secretion of which lubricates the cavity of the oesophagus, thus aiding the passage of the food.

(*c*) The *mucous membrane* lining the inside of the tube consists of a layer of stratified epithelium.

16. *Fundus region of the stomach.* In describing the tissues from without inwards, note the muscular coat of unstriped fibres which consists of two layers. This coat, by its periodic contractions, churns the food while in the stomach and aids its passage to the small intestine.

Next to the muscular coat is a layer called the *submucous coat* which consists of connective tissue, blood-vessels and a few muscle fibres.

The inside coat, or *mucous membrane,* consists of an epithelial cell lining which dips down towards the submucous coat to form the tubular *fundus glands.* The cells of these glands are of two kinds: the *peptic cells* lining the tube, which secrete pepsin, the ferment of the gastric juice, and the *oxyntic cells,* which are larger ovoid cells lying on the outer side of the peptic cells. The oxyntic cells, which stain more strongly than the peptic cells, are believed to secrete the acid of the gastric juice.

17. *Pyloric region of the stomach.* Compare with the previous section and note the absence of the oxyntic cells and the greater development of the peptic cells in the simpler tubular glands.

VII

THE RUMINANT STOMACH

(A) *Stain and mount sections of* (1) *the Omasum and* (2) *the Abomasum of the sheep.*

Sections have been cut by the freezing method and are given out in water.

Stain with haematoxylin and Van Gieson's stain and examine with the naked eye and under the microscope.

(B) *Examine and sketch preserved specimens and demonstration slides of the four compartments of the sheep's stomach.*

18. *Rumen.* Observe in the preserved specimen that the inner surface is studded with rough papillae of various sizes, and in the section that these papillae are lined with stratified epithelium, having a well-developed horny layer. This part of the stomach acts as a store-house for food before rumination.

19. *Reticulum.* In the preserved specimen note that the mucous membrane is thrown into folds having a honeycomb appearance. Observation of the section will show that the folds and base of the honeycomb are studded with small papillae. The folds and papillae are lined with tough stratified epithelium and the larger folds contain a band of muscle. The reticulum acts as a reservoir for water and the more liquid parts of the food.

20. *Omasum.* Observe that the mucous membrane is thrown up into a series of large and small leaves, the faces of which are studded by a multitude of hard papillae. In the section it will be seen that these leaves are lined with thick stratified epithelium having a well-developed horny layer. The large leaves contain two, and the smaller one band of muscle fibres. The omasum completes the trituration of the food by rasping it between its leaves.

21. *Abomasum.* Note that the mucous membrane of the preserved specimen is soft, spongy and smooth. Under the microscope the mucous membrane will be seen to consist of a mass of glands which secrete the gastric juice. The abomasum thus acts as a true stomach. Small nodules of lymphoid tissue may be observed in certain portions of the abomasum.

NOTE. *The rumen, recticulum and omasum have the same general structure as the oesophagus and the abomasum resembles the true stomach.*

VIII

CHEMISTRY OF PROTEINS

Carry out the following tests on the solutions of (*a*) diluted egg *albumin* and (*b*) *gelatin*, and record your results:

(1) *The xanthoproteic reaction.*

To some of the solution in a test-tube, add one-third of its bulk of strong nitric acid, and boil. A *yellow* coloration is developed. Cool under a tap, and cautiously add strong ammonia solution. The yellow colour becomes *orange*.

NOTE. The yellow colour is due to the formation of a nitro-compound of an aromatic substance present in the protein molecule (*e.g. tyrosine, tryptophane,* or *phenyl alanine*). This group is present in egg albumin but exists only in small quantities in gelatine.

(2) *Millon's reaction.*

To some of the protein solution in a test-tube add one half of its bulk of Millon's reagent (a mixture of mercuric and mercurous nitrates in nitric and nitrous acids). Boil the solution. The precipitate or solution develops a brick-red colour.

NOTE. The substance present in the protein to which this reaction is due is *tyrosin*, which is present in albumin but not in gelatin.

(3) *The glyoxylic reaction.*

To some of the protein solution in a test-tube add equal bulk of "reduced oxalic acid." Shake to mix, then cautiously add an equal volume of concentrated sulphuric acid, pouring it down the side of the tube. If a *purple colour* develops at the junction of the fluids *tryptophane* is present.

NOTE. Pure gelatin does not contain tryptophane and so does not react to the test.

(4) *The biuret reaction.*

Treat a little of the protein solution with excess of sodium hydrate, and add a drop of 1 per cent. solution of copper sulphate. A *violet* or *pink colour* is produced.

NOTE. This reaction is given by all substances containing two CONH groups attached to one another or to the same carbon or nitrogen atom.

(5) *The sulphur reaction.*

Boil a little undiluted egg white with some 40 per cent. sodium hydrate for two minutes, and add a drop or two of lead acetate. The development of a *black* coloration indicates the presence of *sulphur* in the *protein* molecule (cystine).

(6) *Molisch's reaction.*

Treat a little of the protein solution with three or four drops of a 1 per cent. alcoholic solution of alpha-naphthol. Mix, run in about 5 c.c. of concentrated sulphuric acid. A violet ring is formed at the junction of the liquids, due to the presence of a *carbohydrate group* in the protein. Compare with cane sugar treated in a similar manner.

Note. Some proteins, such as mucin, secreted by the salivary and other glands and the proteins of young growing tissues contain a carbohydrate grouping.

Note. There is no single simple test by which the presence of a protein may be determined. The protein molecule is a very complex one and the tests that are made for proteins are tests for some particular grouping existing within the molecule. Thus tests (1) and (3) are tests for an aromatic grouping, test (4) is for the linked CONH groups, and test (6) for a carbohydrate grouping.

A reaction to one test is not sufficient to prove the presence of a protein, for example, salicylic acid and benzoic acid give test (1), but these substances will give a negative reaction to test (4).

Some proteins, such as *gelatin*, lack certain component parts of a typical protein and so fail to respond to some of these tests.

IX

THE LIVER AND PANCREAS

(A) *Stain and mount sections of* (1) *the Liver and* (2) *the Pancreas.*

Stain (1) with haematoxylin and Van Gieson's fluid and (2) with haematoxylin and eosin. Examine under the microscope.

(B) *Examine and sketch demonstration slides of*

22. *Liver.* Note that the liver is divided into lobules by bands of connective tissue. These are well marked in the pig but are not so distinct in other animals. Groups of blood-vessels and ducts will be seen at intervals in the connective tissue, each of these groups consists of

(1) A branch of the small thick-walled *hepatic artery* (which brings to the liver fresh blood containing oxygen).

(2) The comparatively large thin-walled *portal vein* (which brings to the liver blood loaded with the products of digestion from the intestine), and

(3) A small *bile duct* (which carries off the secretion of bile to the gall bladder).

The lobules themselves consist of polyhedral *hepatic cells* which have a granular cell substance and often contain a mass of glycogen. These cells are surrounded by a network of blood capillaries and bile ducts.

In the centre of each lobule is a branch of the thin-walled *hepatic vein* by which the blood leaves the liver.

23. *Pancreas.* In appearance the pancreas closely resembles the serous salivary gland, but can be distinguished from it by the presence of clumps of lighter staining protoplasmic cells (islets of Langerhans). The *islet cells* which are well supplied with blood-vessels are believed to have an internal secretion which acts as a regulator of the metabolism of carbohydrates.

Note that the *cells of the alveoli* contain granules which are supposed to be the precursor of trypsin, the most important ferment of the pancreatic juice. The *ducts* are lined by a single layer of epithelium.

X

ACTION OF PANCREATIC JUICE ON PROTEINS: BILE

(A) *Action of pancreatic juice on proteins.*

The mixture of commercial *casein* and commercial *pancreatic* juice given out has been digested for seven days in a flask at 40° C. Carry out the following tests on the mixture:

(1) Test the reaction of the fluid with litmus paper. It is alkaline.

(2) Faintly acidify the solution with dilute acetic acid, and filter. A precipitate (if any) consists of *unchanged casein.* Confirm by applying *xanthoproteic* reaction.

(3) Carefully neutralise the acid filtrate from Exercise (2) by adding dilute sodium carbonate solution. A precipitate indicates *meta-protein.* Filter off precipitate and use filtrate in Exercise (4).

(4) To a portion of filtrate from Exercise (3), add caustic soda and a drop of copper sulphate solution. The biuret reaction, if given, indicates the presence of *albumoses* or *peptones.* If the biuret reaction is given proceed as follows:

(5) To the main bulk of the filtrate from Exercise (3) add solid ammonium sulphate and heat to obtain a saturated solution. Cool under the tap. A precipitate, if any, indicates the presence of *albumose.* Test precipitate which sticks to the tube, by washing with cold saturated solution of ammonium sulphate, dissolving in a little hot water and applying the biuret and the glyoxylic tests.

(6) Test filtrate from Exercise (5) by Millon's test and the glyoxylic test. A reaction indicates the presence of amino-acids.

NOTE. (*a*) In the digestion of proteins by the trypsin of the pancreatic juice the proteins break down in the following order to simpler substances: *protein, meta-protein, albumoses, peptones,* the final end products consisting of *amino-acids.*

(*b*) The method of separating the proteins and their breakdown products depends on the solubility of the protein in various substances and this increases as digestion proceeds.

(B) *Bile.*

(1) *Bile pigments.* Take a few c.c. of fuming nitric acid in a test-tube and by means of a pipette carefully place on the surface of this an equal amount of bile. Shake the tube gently and note the play of colours—yellow, red, violet, blue, green—as the bile pigments become oxidized by the acid (Gmelin's test).

NOTE Bile pigments are formed by the dissociation of haemoglobin which loses first its protein and then its iron constituents.

(2) *Bile salts.* To 50 c.c. of water in a beaker add 10 drops of bile, stir well and allow the fluid to come to rest. Sprinkle some flowers of sulphur on the surface and note that they fall through the liquid to the bottom of the beaker. Repeat the test with a beaker of water alone and note that the particles remain on the surface.

NOTE. Bile salts lower the surface tension of water, and in the intestine assist in the emulsification of the fat.

XI

THE SMALL AND LARGE INTESTINES

(A) *Stain and mount sections of* (1) *the Small Intestine and* (2) *the Large Intestine.*

Stain with haematoxylin and eosin and examine with the naked eye and under the microscope.

(B) *Examine and sketch demonstration slides of*

24. *Small intestine.* On the outer side note the *muscular coat* consisting of two layers, the outer longitudinal and the inner circular. The peristaltic contractions of the muscular coat drive the food along the canal. A *submucous coat* of areolar tissue containing blood-vessels and lymphatics connects the muscular coat with the mucous membrane.

Note that the *mucous membrane* projects into the cavity of the intestine forming the *villi,* whose function is to absorb the digested foodstuffs. The mucous membrane surface is invaginated between the villi to form the *crypts of Lieberkühn,* and below these, in the submucous coat (if the section is from the duodenum), the *glands of Brunner* will be seen. These two glands secrete the intestinal juice which activates the pancreatic secretion and also contains digestive ferments.

25. *Transverse section of villi of the small intestine.* Note that each villus is lined by a single layer of columnar *epithelial cells* through which the digested foodstuffs are absorbed. A number of *goblet cells* which secrete mucin occur among the epithelial cells. The interior of the villus consists of connective tissue in which the numerous *capillaries* (which carry away the absorbed sugar and protein) ramify. In the centre of each villus lies a *lymphatic vessel,* through which channel the absorbed fat is carried to the body.

26. *Large intestine.* On the outside is the *muscular coat* consisting of two layers, the outer longitudinal and the inner circular. The *submucous coat* of connective tissue is well marked and runs up between the ridges into which the mucous coat is generally thrown. The *mucous membrane* contains the *glands of Lieberkühn.* These are similar to those of the small intestine. The epithelium lining the glands and large intestine contains a large number of *mucous cells* (goblet-cells) the secretion of which acts as a lubricant for the passage of the undigested food material.

XII

THE ORGANS OF CIRCULATION

(A) *Stain and mount sections of* (1) *Artery and* (2) *Vein.*

Stain with haematoxylin and eosin and examine under the microscope.

(B) *Examine and sketch demonstration slides of*

27. *Artery.* Note that the walls are comparatively thick and consist of bundles of smooth muscle and elastic fibres arranged circularly. The inner coat is a thin layer of elastic tissue and the lumen is comparatively small.

28. *Vein.* Observe that the wall is thinner than the artery, that the muscular and elastic layer is much reduced and that the lumen is comparatively large.

29. *Heart muscle.* Unlike most involuntary fibres heart muscle is striated, but not distinctly so. Each fibre is short, often branched and has only one nucleus.

(C) *Examine dissected specimen of heart.*

Note the superior vena cava opening into the right *auricle* and the *tricuspid* valve between the auricle and right thin-walled *ventricle.* On the left side note the *pulmonary veins* opening into the *auricle* and the *mitral valve* between the auricle and thick-walled *ventricle.*

(D) *See demonstrations of sphygmograph.*

This is an apparatus for recording the pulse beat. The pressure of the pulse moves a lever which records on a smoked surface moved by clockwork. The curve of pulse pressure shows an upward stroke as the heart beat fills the artery with blood. The curve then falls as the wave of pressure passes and on the down stroke will be noticed the dicrotic notch which is due to elastic recoil of the artery and closure of the aortic valves.

Compare the amplitude and frequency of the pulse beats in different individuals. These vary considerably with the state of health of the animal.

(E) *See demonstrations of Riva-Rocci apparatus for determining blood pressure.*

The principle of the apparatus is as follows: air is pumped into an elastic bag surrounding the arm and the pressure developed recorded by a manometer. Air is forced in until the pressure developed equals that of the arterial pressure; this is determined by the obliteration of the pulse in the radial artery.

XIII

THE ORGANS OF RESPIRATION

(A) (1) *Mount a section of the Trachea which has already been stained in bulk with haematoxylin, and* (2) *stain with haematoxylin and eosin and mount a section of the Lungs.*

Examine the sections under the microscope.

(B) *Stain and mount a thin film of blood* which has been smeared on a coverslip.

Place a drop of a saturated solution of eosin on the coverslip for a few seconds, and blot off the superfluous stain. Place a drop of a saturated solution of methylene blue on the film for a few seconds; blot again, dry well, and add a drop of xylol. Run off the xylol and mount in Canada balsam. Examine under the microscope.

(C) *Examine and sketch demonstration slides of*

30. *Trachea.* Note under the low power of the microscope that a *cartilaginous horse-shoe* occupies the greater portion of the section and that the ends of the horse-shoe are connected by a *muscular band.* The mucous membrane is lined by a layer of *ciliated epithelium* and in its deeper parts consists of connective tissues and small mucous glands.

31. *Lung.* At various places throughout the section observe:

The *bronchioles,* which are lined by ciliated epithelium and have a coat of muscle fibres.

The *alveoli,* which are air sacs, appear as roundish areas of various sizes. Note that the walls of the alveoli are very thin and contain elastic fibres with a *network of blood capillaries.* The alveoli are lined by large flattened cells (pavement epithelium).

32. *Blood.* Note the very numerous *red blood corpuscles* consisting of biconcave discs. They contain the haemoglobin of the blood and convey oxygen to the tissues. The *leucocytes,* or white corpuscles, are of various kinds and are much less numerous. Some contain fine red granules (stained with eosin) and others are clear, but stain slightly with methylene blue; all contain nuclei (stained blue) and some are multinuclear. The number and variety of leucocytes varies considerably according to the state of health of the individual.

XIV

THE BLOOD

(A) *General reactions and properties of blood pigment (haemoglobin) and its derivatives.*

(1) *Oxyhaemoglobin.* Take 5 c.c. of distilled water in a test-tube, add one drop of defibrinated blood, shake vigorously, and examine the spectrum by means of the spectroscope. There are two bands in the green, characteristic of *dilute* oxyhaemoglobin.

Add two more drops of blood and re-examine. The two bands have merged into one wide band, characteristic of *medium* oxyhaemoglobin.

(2) *Haemoglobin.* To a solution of *dilute* oxyhaemoglobin prepared as in (1) add two drops of ammonium sulphide, mix and warm to about 50° C. and examine the spectrum. A *single broad band* in the green will be seen. On shaking vigorously, the characteristic bands of *oxyhaemoglobin* will reappear for a short while.

NOTE. The absorption spectra given by blood pigment and its derivatives form a means of identifying blood in stains or in solution.

The above experiments show that haemoglobin (2) easily absorbs oxygen from the air to form oxyhaemoglobin (1), and easily parts with it again to the tissues (or in (2) to ammonium sulphide).

(3) *Carboxyhaemoglobin.* Examine with the spectroscope the solution of carboxyhaemoglobin, which has been prepared by passing coal-gas through a solution of oxyhaemoglobin. Two well-marked bands will be seen in the green, resembling those of *oxyhaemoglobin.* Attempt to obtain reduced haemoglobin by the method given in (2) *No change takes place in the spectrum.*

NOTE. This ezperiment shows that haemoglobin can combine with carbon monoxide in place of oxygen, and that this combination is very stable. In this state blood cannot carry oxygen and so death results (gas poisoning) from suffocation.

(4) *Methaemoglobin.* To 5 c.c. of water add four drops of defibrinated blood, and two drops of a saturated solution of potassium ferricyanide, and examine with a spectroscope. A well-marked band will be seen in the *red* of the spectrum.

NOTE. By the use of oxidizing agents the oxygen can be firmly fixed to the haemoglobin to form a more stable compound.

(5) *Haemochromogen* (reduced alkaline haematin). Treat a moderately strong solution of oxyhaemoglobin with a few drops of strong NaHO and warm. The colour changes to brown, and examination by the spectroscope shows a faint band in the red. Add a few drops of ammonium sulphide and examine with the spectroscope. Two absorption bands are seen in the green.

Note. Haemochromogen is a breakdown product of haemoglobin in the absence of oxygen and is probably illustrative of the breakdown products that occur between haemoglobin and bile pigments, and of the many derivatives of haemoglobin that are formed in the boiling and salting of meat.

(6) *Preparation of haemin crystals.* Place a drop of defibrinated blood on a glass slide, add a speck of sodium chloride and rub with a glass rod until the salt is dissolved. Evaporate to dryness over a small flame, keeping the slide at least 1 foot away from the flame. Rub the red residue to a fine powder, collect in a heap on the slide, and add a drop of glacial acetic acid on the end of a glass rod. Rub into a paste, place a little of the paste on a clean slide, add a drop of glacial acetic acid, cover with a slip, and cautiously heat over a small flame till it just boils. Run another drop of acetic acid under the slip, allow to cool, and examine microscopically for the characteristic brown rhombic prisms of haemin.

Note. Haemoglobin is composed of a crystalline substance haematin (closely related to haemin), which contains iron, combined with a protein body, globin. This test is frequently used in cases of suspected blood stains.

(B). *The Coagulation of Blood.*

The clotting of oxalate plasma. Dilute 5 c.c. of plasma with 10 c.c. of distilled water and divide into three portions, *A*, *B*, and *C*.

To *A* add a few drops of 1 per cent. calcium chloride and to *B* add a few drops of fibrin ferment (thrombin). Place the tubes in a water bath at 40° C. and observe them at intervals. *A* clots in a few minutes, *B* clots slowly, and *C* does not clot.

Note. Oxalate plasma is prepared by drawing blood into a 1 per cent. solution of potassium oxalate. The corpuscles are removed by a centrifuge.

XV

THE KIDNEY AND SKIN

(A) *Stain and mount sections of* (1) *Kidney and* (2) *Skin.*

The blood-vessels of the kidney have been injected with carmine gelatine; stain with haematoxylin.

The sections of the skin have been cut by a freezing microtome and are given out in water. Commence the process of staining by placing in haematoxylin and afterwards stain with Van Gieson's fluid. Dehydrate and mount as before.

(B) *Examine and sketch demonstration slides of*

33. *Kidney with blood-vessels injected.* Note that the blood-vessels (red injection) form a mass of branching capillaries in the *glomeruli,* which are seen as dark globes lying in the cortex of the kidney. It is supposed that the fluids of the urine are secreted chiefly through the glomeruli. The blood-vessels also form a network around the *secretory tubules,* which are recognised by their darker, slightly granular appearance. The secretory tubules are believed to effect the secretion of the nitrogenous contents of the urine.

The *collecting tubules* consist of rather lighter staining cells and are of comparatively small diameter.

34. *Bladder.* Note the layer of *unstriated muscle fibres* and the internal coat of *stratified epithelium.*

35. *Skin.* Observe that the outer layer or *epidermis,* consisting of epithelial cells, contains several layers; on the outside, a *horny layer* of much flattened cells without nuclei, a *granular layer* often stained deeply with carmine, and an inner layer, the *rete mucosum,* from which the others are formed.

The *dermis* lies beneath the epidermis and consists of elastic tissue, connective tissue, and frequently fat cells.

Sweat glands occur in the dermis as coiled tubes and the ducts can be traced in the epidermis as spiral pores.

36. *Hair follicles.* In a skin section note that the *hairs* lie in the middle of a *follicle* which is formed by a dipping down of the cells of the epidermis and which at the base expands to a *bulb* forming the root of the hair. At the side of the hair follicle and opening into it notice the *sebaceous glands* which consist of masses of polyhedral cells. The secretion of these glands form what is known as the "grease" of wool. Just below these glands a band of *unstriated muscle* is seen and this on contraction erects the hair.

(For *Applied Histology of Skin and Hair* see Practical XXIV.)

XV.

THE KIDNEY AND SKIN

(A) *Cut and mount sections of (1) Kidney, and (2) Skin.*

The blood-vessels of the kidney have been injected with carmine gelatine; stain with hæmatoxylin.

The sections of the skin have been cut by a freezing microtome and are given out in water. Commence the process of staining by placing in hæmatoxylin and afterwards stain with Van Gieson's fluid. Dehydrate and mount as before.

(B) *Examine and sketch demonstration slides of:*

23. *Kidney with blood-vessels injected.* Note that the blood-vessels (red injection) form a mass of branching capillaries in the *glomeruli*, which are seen as dark globes lying in the cortex of the kidney. It is supposed that the fluids of the urine are secreted chiefly through the glomeruli. The blood-vessels also form a network around the *secretory tubules*, which are recognised by their darker, slightly granular appearance. The secretory tubules are believed to effect the excretion of the nitrogenous contents of the urine.

The *collecting tubules* consist of rather lighter staining cells and are of comparatively small diameter.

24. *Bladder.* Note the layer of *unstriated muscle fibres* and the internal coat of *stratified epithelium*.

25. *Skin.* Observe that the outer layer or *epidermis*, consisting of epithelial cells, contains several layers; on the outside, a *horny layer* of small flattened cells without nuclei, a *granular layer* often stained deeply with carmine, and an inner layer, the *rete mucosum*, from which the others are formed.

The *dermis* lies beneath the epidermis and consists of elastic tissue, connective tissue, and frequently fat cells.

Sweat glands occur in the dermis as coiled tubes and the ducts can be traced in the epidermis as spiral pores.

26. *Hair follicles.* In a skin section note that the hairs lie in the *hair follicle*, a tubule which is formed by a dipping down of the cells of the epidermis and which at the base expands to a bulb forming the root of the hair. At the side of the hair follicle and opening into it notice the *sebaceous glands* which consist of masses of polyhedral cells. The secretion of these glands forms what is known as the "grease" of wool. Just below these glands a band of unstriated muscle is seen and this on contraction erects the hair.

(For *Applied Physiology of Skin and Hair* see Practical XXIV.)

XVI

CHEMISTRY OF URINE

The constituents of urine. (Use horse's and human urine.)

(1) Test reaction of human and horse's urine to litmus, and record results..

NOTE. The urine of Carnivora is acid and clear, that of Herbivora is turbid (due to deposition of calcium carbonate), and the reaction is alkaline.

(2) Test for *chlorides* by acidifying a portion of urine with nitric acid, and adding a few drops of silver nitrate solution. A white precipitate indicates presence of chlorides.

(3) To a test-tube nearly full of urine add a little strong ammonia and boil. Filter off the flaky precipitate formed (*phosphates of Ca and Mg*) wash precipitate with water, and dissolve in a few c.c. of dilute acetic acid. Divide the solution into two parts. To one part of the solution add a solution of potassium oxalate. A white precipitate indicates presence of *calcium*. To the other portion add equal bulk of strong nitric acid, about 5 c.c. of ammonium molybdate, and boil. A yellow precipitate indicates the presence of *phosphates*.

(4) Test for sulphates by treating about 10 c.c. of urine with a few drops of strong HCl, and a little barium chloride solution. A white precipitate indicates the presence of *sulphates*.

NOTE. In herbivorous urine phosphates are often absent, but sulphates are abundant.

(5) *Isolation of urea from urine.* (Use human urine.)
Evaporate about 30 c.c. of urine to complete dryness, finishing off the evaporation on a water bath. Turn out the flame and rub the residue with 10 c.c. of acetone. Replace the dish containing the acetone on the water bath, and allow the acetone to boil away to half its bulk. Pour off the acetone on a watch glass, and allow to cool. Crystals of urea separate out as silky needles.

(6) *To demonstrate presence of uric acid in urine.* (Use human urine.)
Treat 50 c.c. of urine with two drops of ammonia and saturate the solution with powdered ammonium chloride. Allow excess of ammonium chloride to settle and pour off the supernatant liquid into another beaker. The liquid contains a gelatinous precipitate of ammonium urate. Filter off the precipitate and transfer it to an evaporating dish. Add two or three drops of strong nitric acid, and evaporate to dryness on a water bath. A *pink* residue is left which becomes *purple* on the addition of dilute ammonia solution.

(7) *To demonstrate the presence of indican.* (Use human and horse's urine.)

Treat 5 c.c. of urine with slightly larger volume of concentrated HCl and about 2 c.c. of chloroform. Add a drop of 3 per cent. potassium chlorate solution and shake. Allow chloroform to settle and examine its colour. *Blue* indicates presence of *indican.* If still colourless add another drop of chlorate solution. If no blue colour be found in the chloroform indican is absent.

Indican is formed from indol which is produced by the bacterial decomposition of tryptophane in the intestine. It is present in horse's urine, but not normally in human except in cases of severe indigestion.

NOTE. Abnormally, in cases of disease, urine may contain albumin (albuminuria), dextrose (diabetes), blood pigments (haematuria), and bile.

XVII

THE NERVOUS SYSTEM

(A) *Examine and sketch demonstration slides of*

37. *Nerve cells from the spinal cord.* Note that the cell has a relatively *large nucleus* and contains numerous *Nissl's granules.* Observe (1) the *axon,* the continuation of which forms the nerve fibre, and (2) the nerve cell processes or *dendrons.*

38. *Medullated nerve fibre.* Note the *axis cylinder* and the *medullary sheath,* consisting of a fatty substance, which surrounds it. The fatty sheath is interrupted at intervals by the *nodes of Ranvier.*

39. *Nerve ganglion.* Observe the *nerve cells* and the *nerve fibres* leaving the ganglion.

40. *Section of spinal cord.* Observe with the naked eye and under the microscope. Note the *grey matter,* more darkly stained, lying in the centre and forming on each side two horns. These consist mainly of nerve cells and non-medullated fibres. The *white matter* consisting chiefly of medullated fibres forms the outer portion of the cord. Note the *roots of the spinal nerves* on each side; the *anterior root* consists of fibres running from the spinal cord (motor nerves) and the *posterior root* of fibres entering the cord (sensory nerves).

41. *Brain.* Note the large number of nerve cells arranged in definite layers just below the surface. The gray matter which contains these cells is on the surface and is thrown up in folds.

(B) *Examine preserved specimens of the brain,* and, proceeding from the *spinal cord* upwards, note: the *medulla oblongata* or spinal bulb (which contains the nerve centre concerned in respiration), the *cerebellum* (the centre which controls co-ordination of muscular movement and equilibrium) and the two *cerebral hemispheres* (which are concerned with consciousness, intelligence and movement).

(C) *See demonstration of muscle-nerve preparation.* The living gastrocnemius muscle of a frog, together with the attached sciatic nerve, has been dissected out and arranged so that the nerve can be stimulated by the passage through it of an electric current. The tendon of the muscle has been attached to a lever so that its movement can be recorded on a revolving drum.

Note that (1) stimulation of the nerve is followed by *contraction* of the muscle which then relaxes again; that (2) a number of stimuli following in quick succession cause *tetanus*—a series of contractions which fuse to form a more long-continued and powerful contraction; and that (3) the muscle if kept in a state of tetanus for some time becomes *fatigued* and gradually relaxes.

XVIII

THE HORSE'S FOOT

(A) *Examine and sketch demonstration slides of*

42. *Bone.* Note the *Haversian canals* which contain blood-vessels, the *canaliculi* or communicating branches between the canals, and the concentric *rings of bony substance* round the canals.

43. *Ossification of cartilage.* Observe, in passing from the end to the centre, that the normal cartilage cells have become arranged in rows and have only a small amount of matrix around them. In this situation bone is eventually deposited.

44. *Hoof of a foetus.* Note the outer layer of horn formed from the compressed epidermal cells. The lower layers of cells in the epidermis are thrown into folds—laminae—and are often pigmented. Between the *horny laminae* of the epidermis lies the connective tissue of the dermis—the *sensitive laminae*—which contain numerous blood-vessels.

(B) *Dissect a horse's foot which has been macerated and examine demonstration specimens.*

Bones. Note the *pedal, navicular* and *coronet,* and observe the function of the navicular in providing a yielding articulation by resting partly on the pedal bone and partly on the coronet.

Soft parts. Note: (1) the *perforans tendon* which passes beneath the navicular and is inserted in the pedal bone; (2) the *lateral cartilages* attached to the wings of the pedal bone, and that these admit expansion of the heels under the influence of the body weight; (3) the *plantar cushion,* a fibro-fatty mass placed between the lateral cartilages at the back of the foot, which absorbs the concussion by its elastic nature; (4) the *sensitive laminae,* which dovetail into the horny portions of the foot and which contain numerous blood-vessels.

Hoof. Note: (1) the *wall,* which forms the main portion of the hoof, is thickest at the toe where wear is greatest, and thinnest at the heel. On the inner surface note the *horny laminae* which dovetail into the sensitive parts of the foot. (2) The *bars* seen on the base of the foot consist of the ends of the inturned wall and allow the wall to yield under pressure. (3) The *frog,* a soft piece of horn which fills up the space between the bars. (4) The *sole,* the concave piece of horn which covers the base of the foot. (5) The *periople ring* at the junction of the wall of the hoof with the skin of the leg; this secretes a varnish covering the external surface of the wall.

XIX

THE DUCTLESS GLANDS

(A) *Stain and mount sections of* (1) *Thyroid and* (2) *Spleen.*

Stain (1) with haematoxylin and Van Gieson's stain, and (2) with haematoxylin and eosin. Examine under the microscope.

(B) *Examine and sketch demonstration slides of*

45. *Spleen.* Note on the outside the *capsule,* consisting of muscular and connective tissue, with its numerous bands passing in to the body of the organ. Periodic contraction of the capsule forces blood out of the organ. The *splenic pulp* consists of lymphoid tissue together with a large number of red blood corpuscles. Round darker masses of tissue—the *malpighian corpuscles*—are seen to be scattered in the pulp. The deep staining is due to the number of leucocytes that have accumulated in this position. The spleen is believed to act as a blood filter and becomes greatly distended in cases of anthrax.

46. *Thymus.* Observe that it is composed of a number of *lobules* separated by connective tissue. Each lobule consists of two portions: (1) the centre part or *medulla,* which consists of loose lymphoid tissue containing the *concentric corpuscles of Hassall*; (2) the external, more deeply staining part or *cortex,* which is composed of lymphatic tissue.

47. *Pituitary.* This gland consists of three portions: (1) the *anterior lobe,* consisting of masses of epithelial cells, which are believed to have an internal secretion influencing growth; (2) the *posterior lobe,* consisting of lighter staining nervous connective tissue cells; and (3) the *intermediate part,* which consists of small granular cells surrounding the neck of the posterior lobe.

48. *Suprarenal.* Note that it consists of a mass of glandular epithelium, the cells of which are packed in rows in the *cortex,* but are arranged irregularly in the *medulla,* which contains blood-vessels. These cells secrete adrenalin, which appears essential for maintaining the muscular tone of the body.

49. *Thyroid.* Observe that the gland consists of a number of roundish *vesicles* filled with a *colloid substance* and lined by a single layer of *cubical epithelium.* Numerous *blood-vessels* run between the vesicles. These carry away from the colloid substance the internal secretion iodothyrin, the absence of which causes cretinism in children and myxoedema in adults.

Pancreas (see Practical IX).

XX

THE MALE REPRODUCTIVE ORGANS

(A) *Stain and mount section of Testis.*

Stain with haematoxylin and eosin and examine under the microscope.

(B) *Examine and sketch demonstration slides of*

50. *Testis.* Observe the *seminiferous* tubules and the *interstitial cells* packed between them. In the tubules note the various stages of spermatogenesis: spermatogonium, spermatocyte and *spermatozoa.* The interstitial cells are believed to have an internal secretion which controls the secondary sexual characters.

51. *Spermatozoa.* Note the head, short cylindrical body and long vibratile tail. Observe also the large numbers in which they occur.

52. *Epididymis.* The convoluted tubes of the epididymis contain *masses of spermatozoa.* The tubes are bound together by connective tissue, contain *involuntary muscle fibres* in their walls and are lined by *ciliated epithelium.*

53. *Vas deferens.* Note the epithelial lining and the very thick muscular walls, the contractions of which drive the semen towards the urethra.

(C) *Examine specimens of the male reproductive organs:*

Note the testis, epididymis, vas deferens, seminal vesicles and prostate. Observe the filiform appendage in the penis of the ram.

(D) *Examine specimens which show the effect of castration* on the horn growth in Herdwick sheep. Note that the removal of the testes arrests horn growth forthwith.

XXI

THE FEMALE REPRODUCTIVE ORGANS

(A) *Hand and razor sections of (1) Ovary and (2) Uterus.*

Stain with haematoxylin and eosin and examine under the microscope.

(B) *Examine and sketch demonstration slides of*

32. *Ovary.* Note the Graafian follicles of various sizes imbedded in the connective tissue stroma, which also contains a number of small rounded interstitial cells.

The small Graafian follicle consists of a large spherical ovum with a well-marked nucleus surrounded by a layer of smaller cells, the follicular epithelial cells. The larger Graafian follicles show a greater development of the follicular epithelial cells, between which fluid (the liquor folliculi) has accumulated. The large accumulation of this fluid causes the follicle to project from the surface of the ovary and ultimately burst, thus setting free the ovum.

33. *Ovary showing corpus luteum.* Observe that the corpus luteum consists mainly of hypertrophied *follicular epithelial cells* and strands of connective tissue containing blood-vessels. The corpus luteum is formed from the Graafian follicle after discharge of the ovum and it is believed to have an internal secretion which controls the nutrition of the uterus and mammary gland.

34. *Fallopian tube.* The wall of the tube consists of two layers of muscular fibres. Note that the mucous membrane is very much folded and is lined by a layer of ciliated epithelium which sets up a current towards the uterus.

35. *Non-pregnant uterus.* Note the two layers of muscular fibres and the mucous membrane lined by a layer of columnar epithelium. The [illegible] in the connective tissue [illegible] and show [illegible] are seen in the [illegible].

36. *Menstruating uterus.* Compare with the last section and note that the mucous membrane is partly stripped. The blood capillaries of the mucosa are engorged with blood.

37. *Female pregnant uterus.* Compare with the two previous sections and note the [illegible] development of the uterine glands. The [illegible] is brought about by the formation of [illegible] in the uterus of those animals which [illegible] internally.

XXI

THE FEMALE REPRODUCTIVE ORGANS

(A) *Stain and mount sections of* (1) *Ovary and* (2) *Uterus.*

Stain with haematoxylin and eosin and examine under the microscope.

(B) *Examine and sketch demonstration slides of*

54. *Ovary.* Note the *Graafian follicles* of various sizes lying embedded in the *connective tissue stroma,* which also contains a number of small epithelial *interstitial cells.*

The small Graafian follicle consists of a large spherical *ovum* with a well-marked nucleus surrounded by a layer of smaller cells, the *follicular epithelial cells.* The larger Graafian follicles show a greater development of the follicular epithelial cells, between which fluid (the *liquor folliculi*) has accumulated. The large accumulation of this fluid causes the follicle to project from the surface of the ovary and ultimately burst, thus setting free the ovum.

55. *Ovary showing corpus luteum.* Observe that the corpus luteum consists mainly of *hypertrophied follicular epithelial cells* and *strands of connective tissue* containing *blood-vessels.* The corpus luteum is formed from the Graafian follicle after discharge of the ovum, and it is believed to have an internal secretion which controls the nutrition of the uterus and mammary gland.

56. *Fallopian tube.* The wall of the tube consists of two layers of *muscular fibres.* Note that the mucous membrane is very much folded and is lined by a layer of *ciliated epithelium* which sets up a current towards the uterus.

57. *An-oestrous uterus.* Note the two layers of *muscular fibres* and that the mucosa is lined by a layer of *columnar epithelium.* The *glands of the mucosa,* which lie in the connective tissue beneath the epithelium, are only moderately developed and show no signs of activity. Only a few *small blood capillaries* are seen in the mucous membrane.

58. *Pro-oestrous uterus.* Compare with the last section and note that the mucous membrane is much swollen. The blood capillaries of the mucosa are congested with blood.

59. *Pseudo-pregnant uterus.* Compare with the two previous sections and note the swollen connective tissue of the mucosa and the very great development of the uterine glands. The pseudo-pregnant condition is brought about by the formation of corpora lutea in the ovaries of those animals which do not ovulate spontaneously.

(C) *Examine specimens of the female reproductive organs.*

Note the ovaries (sometimes containing corpora lutea), Fallopian tubes, uterus, cervix, vagina and vulva.

(D) *Examine demonstration slides which illustrate the influence of the ovary on the uterus:*

(1) Uterus of rat after ovariotomy, showing atrophy.

(2) Kidney of rat into which an ovary has been transplanted.

(3) Uterus of rat after ovary has been transplanted. The uterus is normal.

XLII

THE MAMMARY GLAND

(a) *Fresh and teased sections of the Mammary Gland.*

Stain with haematoxylin and eosin and examine under the microscope.

(1) *[illegible] and their [illegible] stages of* [illegible]

(a) *Mammary gland in the developing state.* Note the ducts and the acini which are being formed in lobules as outgrowths from the ducts. The alveoli are completely filled with epithelial cells and are embedded in a mass of connective and adipose tissue. Growth of the gland takes place slightly at puberty, but mainly during the first pregnancy.

Mammary gland actively secreting. Observe that the gland consists almost entirely of alveoli and that only a little connective tissue is present between them. The appearance of the alveolar epithelium depends on whether the milk has been secreted or not.

(1) Where secretion has taken place the lumen is distended with milk and the cells lining the alveoli appear flattened.

(2) Where the gland has been emptied of milk the alveoli are collapsed and the epithelial cells, often showing two nuclei, project into the lumen.

(c) *Mammary gland at the end of lactation.* Note that the alveoli have undergone involution and that there is a large amount of connective tissue between the lobules.

(d) *Mammary gland stained with Sudan III.* The fat globules [illegible] are seen in the cells of the alveolus.

Note.—The mammary gland differs from many other glands in [illegible] that the [illegible] considerably with the [illegible] of the [illegible].

(b) *[illegible] specimen of a cow's udder which has been injected* through the nipple with plaster of Paris. Note the [illegible] which shows large milk cisterns, the [illegible] of the [illegible] and the [illegible] which [illegible].

(c) *[illegible]* a cell of [illegible] from a black pig.

The [illegible] is [illegible] in the [illegible] of the [illegible] and [illegible] from the [illegible] of the [illegible]. The [illegible]

[illegible] the female reproductive organs.

Note the [illegible] Fallopian tubes, [illegible].

[illegible] the [illegible] of the [illegible].

(1) [illegible]

(2) [illegible]

(3) [illegible] has been [illegible]. The [illegible]

XXII

THE MAMMARY GLAND

(A) *Stain and mount sections of the Mammary Gland.*

Stain with haematoxylin and eosin and examine under the microscope.

(B) *Examine and sketch demonstration slides of*

60. *Mammary gland in the developing state.* Note the *ducts* and the *alveoli* which are being formed in lobules as outgrowths from the ducts. The alveoli are completely filled with epithelial cells and are embedded in a mass of *connective* and *adipose* tissue. Growth of the gland takes place slightly at puberty, but mainly during the first pregnancy.

Mammary gland actively secreting. Observe that the gland consists almost entirely of alveoli and that only a little connective tissue is present between these. The appearance of the alveolus depends on whether the milk has been secreted or not.

61. Where secretion has taken place the *lumen* is distended with *milk* and the cells lining the alveoli appear flattened.

62. Where the gland has been emptied of milk the alveoli are collapsed and the epithelial cells, often showing two nuclei, project into the lumen.

63. *Mammary gland at the end of lactation.* Note that the alveoli have undergone involution and that there is a large amount of connective tissue between the lobules.

64. *Mammary gland stained with Sudan III.* The fat globules, stained red, are seen in the cells of the alveolus.

NOTE. The mammary gland differs from many other organs in the body in that its appearance varies considerably with the reproductive state of the animal.

(C) *Examine specimen of a cow's udder* which has been injected through the nipple with plaster of Paris. Note the glandular tissue, milk ducts, large milk cistern, the ducts of the nipple and the sphincter muscles which close it.

(D) *Pigmented mammary region* in a side of bacon from a black pig.

The black pigment is contained in the ducts of the mammary gland and originates from the black pigment of the skin. The pigment disappears with the onset of lactation.

(3) *Museum specimens of the female reproductive organs.*

Note the ovaries (containing corpora lutea), Fallopian tubes, uterus, cervix, vagina and vulva.

(4) *Museum demonstration slides which illustrate the influence of the ovary on the uterus:*

- (1) Uterus of rat after ovariectomy, showing atrophy.
- (2) Uterus of rat in which an ovary has been transplanted.
- (3) Uterus of rat after ovary has been transplanted. The uterus is normal.

XXII

THE MAMMARY GLAND

(A) *Stain and mount sections of the Mammary Gland.*

Stain with haematoxylin and eosin and examine under the microscope.

(B) *Examine and sketch demonstration slides of*

60. *Mammary gland in the developing state.* Note the *ducts* and the *alveoli* which are being formed in lobules as outgrowths from the ducts. The alveoli are completely filled with epithelial cells and are embedded in a mass of *connective* and *adipose* tissue. Growth of the gland takes place slightly at puberty, but mainly during the first pregnancy.

Mammary gland actively secreting. Observe that the gland consists almost entirely of alveoli and that only a little connective tissue is present between these. The appearance of the alveolus depends on whether the milk has been secreted or not.

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62. Where the gland has been emptied of milk the alveoli are collapsed and the epithelial cells, often showing two nuclei, project into the lumen.

63. *Mammary gland at the end of lactation.* Note that the alveoli have undergone involution and that there is a large amount of connective tissue between the lobules.

64. *Mammary gland stained with Sudan III.* The fat globules, stained red, are seen in the cells of the alveolus.

NOTE. The mammary gland differs from many other organs in the body in that its appearance varies considerably with the reproductive state of the animal.

(C) *Examine specimen of a cow's udder* which has been injected through the nipple with plaster of Paris. Note the glandular tissue, milk ducts, large milk cistern, the ducts of the nipple and the sphincter muscles which close it.

(D) *Pigmented mammary region* in a side of bacon from a black pig.

The black pigment is contained in the ducts of the mammary gland and originates from the black pigment of the skin. The pigment disappears with the onset of lactation.

(E) *Examine specimens which illustrate the development of the mammary gland in the rabbit.*

(1) *Virgin*, which is small and consists mainly of a few ducts.

(2) *Pseudo-pregnant* (10 days), which shows growth of alveoli.

(3) *Pseudo-pregnant* (23 days), which shows gland commencing to atrophy.

(4) *Pregnant* (25 days), which illustrates the growth in thickness of the gland.

(F) *Examine photographs which show the effect of an injection of pituitary extract on the secretion of milk.*

XXIII

PREGNANCY

(A) *Examine with the naked eye and under the microscope the germinal area of a fowl's egg in which incubation has started.*

(1) *About* 18 *hours.* Note the primitive groove and the medullary folds from which the neural canal is formed.

(2) *About* 68 *hours.* Note the neural canal, the patches of mesoderm forming the rudimentary vertebrae, the heart and blood-vessels with their extensions over the germinal area.

(B) *Examine the placenta, membranes, and umbilical cord of a foetal sheep.*

Note the attachment of the *foetal membranes* to the *cotyledons* of the uterus and observe the two foetal membranes—the *amnion,* closely surrounding the foetus, and the *allantois,* forming the outer envelope. Observe the course of the foetal blood-vessels passing from the umbilicus to the cotyledons and returning again to the umbilicus.

Compare the different types of placenta that occur in the dog (zonary), rabbit (discoidal), and pig (diffuse).

(C) *Examine and sketch demonstration slides of*

(1) *Placenta of rabbit.* Note the *foetal tissues containing blood-vessels* which dovetail with the *mucous layer of the uterus,* the cells of which have become transformed to *decidual cells* surrounding the *maternal blood-vessels.* The active uterine *glands,* which form the uterine milk, are seen at the borders of the placenta.

(2) *Placenta of sheep* (cotyledon). Note the *crypts of the cotyledons* containing the *foetal villi.* Between the crypts observe the maternal decidual cells and blood-vessels.

(D) *Examine specimens which illustrate some of the factors controlling fertility.*

(1) *Section of ovary showing degenerate follicles.* Note that frequently the follicle is filled with blood from the ruptured vessels, that the ovum is not circular but shrivelled, and that the follicular cells are disappearing.

(2) *A litter of embryos from the uterus of a sow.* Note that some of these undergo a very limited degree of development and become mummified.

(E) *Examine an apparatus for artificial insemination.*

XXIV

MEAT AND WOOL

(A) *Examine specimens which illustrate various factors which influence the quality of meat.*

(1) *Marbling.* Note that marbling consists of the deposition of fat in the connective tissue between the bundles of muscular fibres. The amount of marbling depends on the degree of fatness of the animal and its breeding and age, as well as varying in the different muscles of the body.

(2) *Tenderness.* This depends partly on the amount of connective tissue between the muscle fibres and bundles and this diminishes with the distance from the attachment of the muscle. The connective tissue when present in large quantities makes the meat tough. Tenderness is also dependent on the denseness of the muscle fibres and thickness of the cell walls, which factors vary with use by and with the age of the animal.

(B) *Examine specimens which illustrate various factors influencing the quality of wool.*

(1) *Diameter of wool fibre.* Compare Herdwick and Merino wool and examine sections of the skin of these two breeds. The wool and hair follicles of the latter are much finer than those of the former.

(2) *Lustre.* Compare Leicester and Shropshire wool. The lustre of the former is due to the form of the cuticle of the wool fibre, the scales of which are closely pressed together and so reflect the light. Non-lustre wool has protruding scales. The presence of grease in the wool probably adds to the lustre.

(3) *Crimp.* The wool fibre is not straight but grows in wavy manner. The frequency of these waves—called crimp—varies in the different breeds of sheep. Compare Merino wool (about 20 crimps to the inch) with Kent wool (about 5 crimps to the inch).

(4) *Length of staple.* Compare the wools of the Shropshire and Merino (short wool) with those of the Lincoln and Blackface breeds (long wool). The length of the staple varies in different parts of the body.

(5) *Evenness of staple.* Compare the fibres of even thickness in Merino wool with the fibres of various sizes and colours in Herdwick wool. Kemps or hairs in the wool cause defects in the cloth when the wool is manufactured.

(6) *Amount of yolk present.* Examine Merino skin and wool and compare with Herdwick skin and wool. Note that the large number of sebaceous glands in the Merino skin is associated with more grease in the wool.

(7) *Soundness of the wool fibres.* Weak areas may occur in the wool fibre. These may be due to disease or malnutrition of the sheep during the period at which the wool was grown.

(8) *The portion of the fleece from which the sample was taken.* The finest wool usually occurs over the shoulders and the coarsest is found on the legs, under the belly and over the britch. The wool fibres are long on the back, but short under the belly. The distribution of the coarse and fine fibres varies with the breed.

(C) *Examine slides which show the effect of scalding on the skin of a pig.*

Note that the stratified epithelium and hairs of the epidermis have separated completely from the tissues of the dermis.

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